Remark as to amendment of Specification

In preparing the present Reply Applicants became aware of an inadvertent typographical error in paragraph 0123, and respectfully request entry of the amendment herein, to correct this error. It is clear from the context of the paragraph that Figure 12 is referred to in this paragraph, and not Figure 11.

ARGUMENTS FOR TRAVERSING THE LACK OF UNITY OBJECTION

The present communication contains 2 Annexes which provide evidentiary support for traversal arguments:

-Annex I Page 267 (abstract) of a scientific publication regarding the stability of hGC in relation with pH.
Grabowski GA, Dagan A." Human lysosomal beta-glucosidase: purification by affinity chromatography." Anal Biochem 141: 267-279, 1984
-Annex II Scientific publication published by the applicants on Plant Molecular Biology (2005) 57:101-113:
Serena Reggi et al "*Recombinant human acid beta-glucosidase stored in tobacco seeds is stable, active and taken up by human fibroblasts*".

Annex I discloses the reduction of half life (and hence the loss of enzymatic activity) of hGC in relation to increasing pH values.

Annex II discloses the impossibility of producing lysosmal enzymes in seeds using a 35S promoter, as well as the functionality of the system disclosed in the application. Particular attention should be given to pages 105-108 (results) where experiments proving the inactivity of the 35Spromoter in seed are published.

In reply to the objection raised by the Patent Office concerning the lack of unity of invention, the Office communication mailed 08/22/2006 has raised a lack of unity objection on the present application, defining 4 groups that do not relate to a general inventive concept because allegedly lacking the common essential feature for lack of novelty of the same.

The Office communication states as the technical feature linking groups I to IV "the expression of a lysosomal enzyme in the seeds of a plant".
According to the Office communication, said technical feature is anticipated by Radin et al. in WO97/01353 from now on referred to as D1. This legal conclusion is disputed by the evidence and arguments provided herein.
It is noted that D1 corresponds to US 5,929,304 already cited and discussed in the present application in pages 1 and 2, starting from paragraph 0014 to paragraph 0019.

Initially, Applicants object to said legal conclusion for the following underlying scientific reasons:

1.1 Ineffectiveness of inducible promoters in seed.
Due to the fact that the Prior Art discloses expression of hGC in leaf with inducible promoters, it is essential to point out that inducible promoters are not suitable for in-seed expression. In fact, **said promoters, in order to be functional, must be located in a physiologically active tissue** (such as leaf) and hence may be activated by the inducing molecule.
The seed, being a storage tissue, is a dormant tissue with a water content of about 12-14% that prevents physiological activity, in particular for the present argument activity needed for induction of inducible promoters, until germination.

Hence, the **inducible promoters of the cited prior art are unsuitable *a priori* for in-seed production of desired molecules**.

1.2 Ineffectiveness of 35S promoter in seed.
Figure 12 of the present application proves without doubt the ineffectiveness of the constitutive promoter 35S CaMV for the in-seed expression of hGC.
Said ineffectiveness is further proved in annex I, page 105, see "results" and page 106, second paragraph.

1.3 Enzymatic activity of hGC in relation to pH values.
The state of the art and, in particular, Annex I of the present communication, discloses and proves the shortening of the hGC half life (and hence the loss of enzymatic activity) with

the increase of pH values. As stated in the abstract of Annex I, "*The enzyme had a half life of 65 and 30 min at 50°C and pH 5.0 and 6.0 respectively*". Hence, passing from pH 5.0 to pH 6.0 the half life of the enzyme (expressing the enzyme functionality) is reduced by more than 50%. It is well known in the art that hGC loses completely the enzymatic activity at pH 7.0 even at 37°C.

The extreme importance of the pH for the solubilization and extraction of the enzyme in order to maintain the same in an active form is particularly relevant as explained in point 2.5 of the present communication where it is clear that the enzyme produced in the prior art with the MeGa promoter, cannot be extracted and solubilized from the leaf in an enzymatically active form..

The above scientific evidence supports that legal conclusion that it is incorrect to require restriction based on Radin et al., because Radin et al. does not teach the technical feature that links the claims of groups I-IV, these groups being established by the Patent Office. This is because Radin et al. use promoters and systems comprising such promoters that are non functional in-seed. Thus Radin et al. neither effectively teach nor anticipate the claims because Radin et al. has not achieved in-seed expression of introduced enzymes, as is described and claimed in the present application. This should be taken into account by the Patent Office while considering the following arguments.

2.WO 97/10353-D1

D1 is a family member of the US application 5,929,304 cited and discussed by the Applicant in pages 3-5 of the description, paragraphs 0014 to 0019 of the publication. D1 describes the expression of some lysosomal enzymes in tobacco plants. The enzymes are produced essentially in leaf by plants transformed via the use of vectors containing the inducible MeGa promoter (deriving from the tomato HMG2 promoter) or the cauliflower mosaic virus (CaMV) 35S promoter.

2.1 D1, although allegedly claiming also an in-seed expression with the 35S promoter or with several inducible promoters, only demonstrates the use of the promoter 35S and the inducible promoter MeGa for the in-leaf expression of IDUA and the use of the sole MeGa promoter for the in-leaf expression of GC and never demonstrates the possibility of an in-seed expression of said enzymes.

The cited promoters, however, are non functional in-seed as explained in points 1.1 and 1.2 above, consequently do not meet the limitations of the independent claims herein, and therefore the teachings of Radin et al. do not provide, in a technical or in a legal sense, any technical feature that links the claims of groups I-IV.

2.2 With respect to the enzymatic activity of the obtained enzymes it is noted that the MeGA promoter needs an activation time, at room temperature, ranging between 12 and 24 hours in order to obtain high amounts of transcription. However, said activation time, that certainly determines production of rGC, allows as well the degradation of the protein as said enzyme degrades at room temperature. In order to overcome this degradation problem, the incubation times should be reduced, and that would lead to a very small production. As shown in D1, page 40, in which reference is made to figure 4, the maximum level of mRNA is in fact at 24h after activation. Thus, use of the MeGA promoter does not provide the teachings of the present claims, and therefore Radin et al. cannot properly be stated to provide, in a technical or in a legal sense, any technical feature that links the claims of groups I-IV.

2.3. It is also noted that, the only expression demonstrated in D1 is in-leaf and that the in-leaf expression leads to the expression of an enzyme comprising N-linked glycanes such as alpha 1-3 fucose and beta1-2 xylose that are undesirable in enzymes for therapeutical uses as also stated in D1 page 32, line 12. Thus, the expression demonstrated in D1 does not provide the teachings of the present claims, and therefore Radin et al. cannot properly be stated to provide, in a technical or in a legal sense, any technical feature that links the claims of groups I-IV.

2.4. Another serious problem related to the in-leaf production lies in the purification of the enzyme obtained. In fact, although seeds show a very low glucosidasic activity (about 1% of the activity of the recombinant), leaves show a high glucosidasic activity (about 40% of the recombinant according to Fig. 6 at 12 h), said activity rendering very difficult the evaluation of the recombinant lines and the subsequent purification of the protein (cfr. page 42, line 15). Thus, D1 does not provide the teachings of the present claims, particularly with regard to in-seed production of lysosomal enzymes in enzymatically active form at and above the claimed concentrations.

2.5 Furthermore, the in-leaf production with the system described in D1, has another serious inconvenience related to the fact that the enzyme produced is associated with membranes and hence insoluble (cfr. Tab 1 page 43 of D1), therefore requiring solubilization conditions (i.e. sonication, freezing-defrosting cycling) that lead to the inactivation of the enzyme. It is herein noted that the enzyme is, in fact particularly delicate and the room temperature activation for several hours, as required by the activation of the MeGA promoter, and the purification procedures required afterwards bring to the loss of a large part of the enzymatic activity. Thus, D1 does not provide the teachings of the present claims, particularly with regard to in-seed production of lysosomal enzymes in enzymatically active form at and above the claimed concentrations.

Moreover, **the solubilization at pH 7.0** (cfr. page 43, line 32) **leads to the complete loss of enzymatic activity** (see point 1.3 above). In the Applicant's experience, the enzymatic activity of the protein is completely lost at pH values higher than 5.8-6.0. The protein accumulated in-seed in accordance with the present invention, on the contrary, is completely solubilized at pH 5.5 in a buffer that ensures the maintenance of the enzymatic activity.

It is also noted that in point 6.3 of D1, pages 44-46, the technique described for the purification of the enzyme would lead, in the opinion of the Applicant, to an inactive enzyme. It is the opinion of the Applicant, as person skilled in the art, that the activity reported in D1 is due to the gycosidic activity of the plant's endogenous enzymes as with the extraction protocol described (cfr. D1 point 6.2.6 page 43), that uses a buffer at pH 7.0, it is impossible to maintain the glycosidic activity of the human protein.

4. It is also noted that the Western Blot data reported in Figure 5B are very unlikely to be derived by the use of the protocol 6.2.6. described in D1 (page 43) because if the lanes 1, 2, 3 etc. correspond to activation times such as 0, 2, 4, etc. hours post-MeGA the kinetics of accumulation therein reported is inconsistent with the ones reported in literature for the MeGA promoter.

Also, the data reported about the IDUA protein, better demonstrate the long activation times required for the MeGA promoter (8-27 h, cfr. fig 14A) with a pattern similar to another MeGA-driven construct, hGC:FLAG, as stated by the authors of D1 in page 53, lines 16-18.

These data inconsistencies, and the more probable long activation time required for MeGA-driven constructs, provide additional evidence that it is not proper to use Radin et

al. as a reference allegedly providing a common inventive concept linking the claims of groups I-IV. This is because MeGA constructs are neither shown to be effective in seeds, nor are expected, based on a reasonable understanding of the data, to be effective in seeds to achieve the results that are demonstrated in the present application, where these results support the present claims.

5. Moreover, considering that the comparison between the IDUA-9 obtained from tobacco lines and the one produced in CHO shows that the two migrate in slightly different manners (cfr. D1, page 53, lines 26-33), the glycosylation of the protein produced in-plant is not demonstrable due to the different molecular weights together with the glycosylation characteristics (i.e. presence or absence of xylose and or fucose). Further, according to the data shown in D2, enzymes produced in leaf do contain the above-noted N-linked glycanes and hence are unsuitable for therapeutic purposes. The adds further evidence of the failure of Radin et al. to provide, in a technical or in a legal sense, any technical feature that links the claims of groups I-IV

The relative inefficiency of the in-leaf system is acknowledged also by the authors in page 55, lines 6-9 in which they declare, "the extracts showed IDUA activity at relatively low levels".

Moreover, as declared in D1 page 54, lines 27-36, in relation to IDUA but extendable to the lysosomal enzymes of D1, the main problems with said enzymes lies in the functionality of the enzyme once purified, functionality that, for the lysosomal enzymes produced in-leaf, has not been demonstrated. This and the points immediately above support Applicants' assertion that D1 does not provide the teachings of the present claims, particularly with regard to in-seed production of lysosomal enzymes in enzymatically active form at and above the claimed concentrations.

Concluding Points

Summarising, D1 discloses a process for the production of human lysosomal enzymes in-leaf under the control of inducible promoters or under the control of the 35S promoter. No correct glycosylation is described for the produced enzymes (but the results of D2 show the presence, in the enzymes produced in-leaf, of undesirable N-linked

glycanes) and the enzymes produced are membrane linked and thus need purification treatments that are incompatible with the enzyme activity of the purified enzymes. Furthermore, the allegedly claimed level of production, which is nevertheless lower then the one shown in the present application, seems not compatible with the protocols disclosed in D1.

Finally, although the D1 (Radin et al.) document contains claims of a method that include alleged recovering a lysosomal enzyme from a transgenic plant organ where the "organ" is a seed ('seed' being merely one of a number of listed plant parts), the protocols and the promoters indicated in D1 are **not compatible** with in-seed expression, as demonstrated for the inducible promoters (cfr. point 1.1 of the present communication) and the 35S promoter (see Fig. 12 and paragraph 0123 of the present application and annex I). No particular interest is demonstrated in D1 for the in-seed expression nor there is in D1 a disclosure or indication of the tools that would lead to said expression and of the better qualities (i.e. higher production, solubility, stability, correct glycosylation pattern with no xylose or fucose residues) of the enzymes produced in-seed in comparison to the ones produced in-leaf, that would be obtained with such expression. Thus, for the reasons above, taken singly or in any combination, it is improper for the Patent Office to make the assertion that Radin et al. "teach the expression of a lysosomal enzyme in the seeds of a plant," and it is improper to base a restriction requirement requiring an election of a claim group based on this reference, as it does not teach a technical feature linking the invention of claim groups I-IV. Thus, the restriction requirement is improper and traversal, requested herein, is proper.

Further as to particular claims, including dependent claims, is also noted that although the Office communication claims that D1 provide the necessary tool for the in-seed expression, said document, does not indicate nor suggest any of the promoters and of the leader sequences selected by the applicant as suitable for the expression of an enzyme as delicate as GC.

More importantly, D1 does not indicate in any way that a production of said enzymes in-seed would solve all the drawbacks reported above for the in-leaf expression. There is nowhere stated in the art cited that the expression in seed would have led to the production of stable, soluble and active lysosomal enzymes, said enzymes being particularly advantageous for therapeutical purposes as their glycosylation pattern does not include xylose and/or fucose residues.

Hence the applicant, not only produces the enzymes in an new and inventive way, but also solves all the problems related to the in-plant production of said enzymes reported in the state of the art, by selecting the in-seed expression without any previous indication in the state of art that said selection would solve the problems encountered in the in-leaf expression.

It is further noted that Croptech, the applicant of the cited PCT application with Radin et al. inventors, was not commercially successful in its efforts to produce an enzyme expressed in a plant, and filed for bankruptcy in 2003. See http://biotechcareercenter.com/CropTech.html. This provides further evidence of the limitations, as opposed to scope and effectiveness, of the methods of Radin et al.

Having amply demonstrated that Radin et al. do not teach a common technical feature of the claims of groups I-IV, traversal is proper and hereby respectfully requested.

The Examiner is invited to call the undersigned attorney if there are any issues regarding this response that may be resolved via telephone conference.

Respectfully submitted,

Joseph Fischer
Patent Attorney, Registration No. 51,210
Mailing Address and Phone/Fax numbers:
Beusse Wolter Sanks Mora & Maire, P.A.
390 N. Orange Avenue, Suite 2500
Orlando, FL 32801
Telephone: 407-497-7437
Fax: 407-926-7720